REMARKS

Applicants and Applicants' new representative thank the Examiner for speaking with Applicants' new representative on June 28, 2006, to clarify the restriction requirement and subsequent election of a group and species for prosecution.

Applicants have amended claims 27 and 31; cancelled claims 28-30 and 32-36; and added new claims 37-69 as set forth above. Specifically, amended claims 27 and 31 now require that the chelating agent, M, is BX_2 in which X is a halide; that each R^1, R^3, R^4, and R^6 is independently selected from the group consisting of: H; a substituted or unsubstituted, saturated or unsaturated, cyclic, moiety; a substituted or unsubstituted, saturated or unsaturated, heterocyclic moiety; or a substituted or unsubstituted, saturated or unsaturated, straight or branched chain alkyl or acyl moiety; and that each R^2 and R^5 is independently selected from a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom. New claims 37 through 46 recite certain dependent features of the pharmaceutical composition of claim 27; new claims 47 through 57 recite compounds; and claims 58 through 69 recite certain dependent features of the methods of using the compounds for photodynamic therapies. No new matter has been introduced in the foregoing amendments. Support for the amendments can be found in the specification at pages 3 and 5-7.

Rejoinder

Applicants acknowledge the rejoinder of the method claims if the pharmaceutical composition and compound claims are subsequently found allowable. For completeness, Applicants have added new method claims 58 through 69 which recite certain dependent features of the methods of using the compounds for photodynamic therapies. Applicants respectfully request that the Examiner consider and rejoin amended claim 31 and new method claims 58 through 69, when the pharmaceutical composition and compound claims are subsequently found to be allowable.

Objection

The Examiner has objected to the specification for failing to include a detailed description of the drawings. Accordingly, Applicants have amended the specification to include a brief description of the drawings.

Rejections

I. 35 U.S.C. § 102

Claims 27 and 28 stand rejected under 35 U.S.C. § 102 as being anticipated by U.S. Patent No. 5,446,157 by Morgan et al. (the "'157 Patent"). In making this rejection, the Examiner states that the '157 Patent discloses a compound in which M is BF_2, R^2 and R^5, are hydrogen, and R^1, R^3, R^4, and R^6 are alkyl groups. For at least the reasons stated below, Applicants submit that the amended claims are patentable over the '157 Patent.

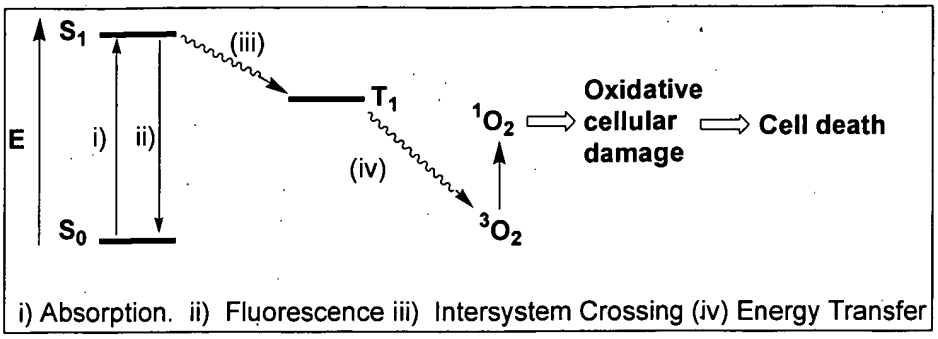
As amended, independent claims 27 and 47 require that each R^2 and R^5 is independently selected from a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom. Thus, the compounds in the '157 Patent do not anticipate the amended claims since the specific R^2 and R^5 groups on the compounds at column 30 of the '157 Patent are hydrogen and not a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom, as required by the amended claims. Accordingly, Applicants request that the rejection of claim 27 and the claims depending therefrom be withdrawn.

II. 35 U.S.C. § 103

Claims 27-30 stand rejected under 35 U.S.C. § 103 as being obvious over the '157 Patent discussed above; U.S. Patent No. 5,326,692 by Brinkley et al. (the "'692 Patent"); and JP Abstract JP 11092479 to Hirosuke, T. (the "JP Abstract"). For at least the reasons stated below, Applicants submit that the amended claims are patentable over the '157 Patent, the '692 Patent, or the JP Abstract, whether taken alone or in combination.

Photodynamic Therapy

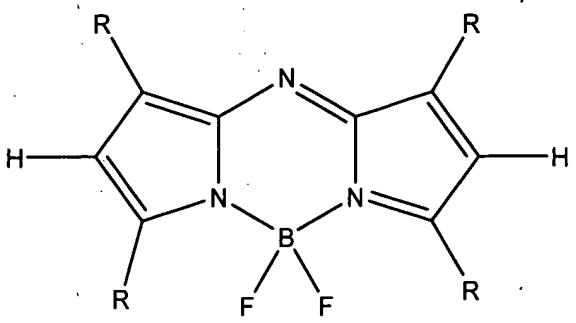
Photodynamic therapy (PDT) is a non-invasive technique for the treatment of a variety of solid tumor types by administering a photosensitizing compound, followed by illumination of the tumor with light. See for example page 1, line 8 through page 2, line 27. In general, the efficacy of a photodynamic therapy (PDT) agent can be governed by at least the following three factors i) the photosensitizer localization, ii) the extent of light activation of the photosensitizer in vivo (determined by the absorption characteristics of the photosensitizer), and iii) the efficiency of an intersystem crossing (ISC) from the first excited singlet state to the triplet state of the photosensitizer (see Figure below).



An electronic transition from a singlet (S1) to a triplet (T1) excited state within a molecule is a spin forbidden process and as such occurs very inefficiently. In order for a transition between states of different spin multiplicities to occur effectively, a spin-orbit perturbation is required within the molecule to facilitate ISC. The compounds in which each R^2 and R^5 is independently selected from a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom, exhibit enhanced spin-orbit perturbations; thereby providing a dramatic increase in the degree of spin-orbit coupling and, as a consequence, the efficiency of populating the T1 excited state. The final step of the singlet oxygen generation process is an energy transfer from photosensitizer triplet state (T1) to ground state oxygen. The rate of production of singlet oxygen, which is the key cytotoxic agent involved in the PDT process, increases when each R^2 and R^5 is independently selected from a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom. It should be noted that compounds in which each R^2 and R^5 is independently selected from a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom could potentially promote non-radiative internal back-conversion from T1 to the ground state S0 or inhibit the photosensitizer triplet to ground state oxygen energy transfer. These competing pathways can give rise to loss of the excited state energy of the photosensitizer without the generation of singlet oxygen. As such, compounds in which each R^2 and R^5 is independently selected from a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom might promote an S1 to T1 intersystem crossing without necessarily resulting in enhanced singlet oxygen production. This effect may be attributed to a shortening of the triplet lifetime as a result of the heavy-atom effect. Surprisingly, this negative impact was not observed and hugely increased level of singlet oxygen and remarkable improvements in efficacy were recorded for compounds in which each R^2 and R^5 is independently selected from a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom.

A. the `157 Patent

In making the obviousness rejection over the `157 Patent, the Examiner states that the `157 Patent discloses compounds useful as photochemical agents in photodynamic therapy techniques and identified an azapyrromethene-BF_2 complex, Compound 26 in Example XI, scheme 2,



in which R represents an alkyl. (See the small genus of compounds at column 30, line 49 through column 31, line 10.) See also the Official Action pages 8-9.

The `157 Patent, however, does not describe or disclose specific compounds in which each R^2 and R^5 is independently selected from a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom, as required by the amended claims. Moreover, Applicants submit that one skilled in the art would not be motivated by the `157 Patent to replace the hydrogen groups with a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom with an expectation of increasing the effectiveness of PDT by increasing the population of T1 and increasing the generation of ground state oxygen while limiting the non-radiative internal back-conversion from T1 to the ground state S0 and the inhibition of the photosensitizer triplet to ground state oxygen energy transfer. Rather, the `157 Patent is concerned with "identifying photosensitizing chemicals which are useful in photodynamic therapy characterized by reduced triplet-triplet (T-T) absorption ...." See column 3, lines 59-61.

Despite the Examiner's reliance on the broad genus of compounds at column 4, lines 55 through column 5, line 44, the `157 Patent teaches a much narrower generic description of azapyrromethene compounds at column 5, line 42 and lines 54 to 68; an even narrower genus of species in Example XI; and preferred compounds, at column 35, lines 40-44, in which R_{16} and R_{19} are hydrogen, $NaSO_3$ or C_nH_{2n+1}, all of which lack a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom at the R^2 and R^5 equivalent

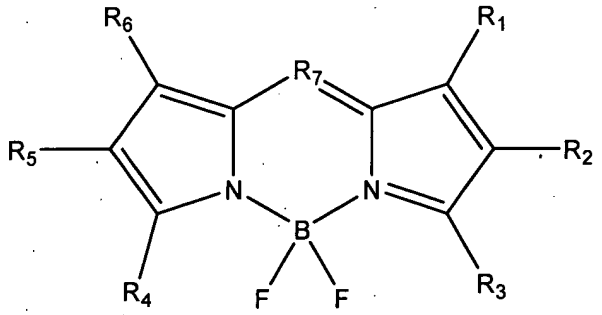
positions. Additionally, the `157 Patent does not disclose any specific examples of compounds in which the substituents at the R^2 and R^5 equivalent positions are a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom.

Taken as a whole, the `157 Patent does not teach or suggest selecting R_{16} and R_{19} from the generic list of substituents at column 5, lines 7 through 35 to be a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom or modifying the R^2 and R^5 equivalent positions of the azapyrromethene-BF_2 complex in Example XI to be a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom in order to reduce triplet-triplet (T-T) absorption, let alone selecting certain substitutes to produce compounds exhibiting increased population of T1 and increased the generation of ground state oxygen while limiting the non-radiative internal back-conversion from T1 to the ground state S0 and the inhibition of the photosensitizer triplet to ground state oxygen energy transfer.

For at least the foregoing reason, Applicants submit that the amended claims are non-obvious over the `157 Patent.

B. The `692 Patent

In making the obviousness rejection over the `692 Patent, the Examiner states that the `692 Patent discloses compounds of the formula



See the Official Action pages 9-10.

The `692 Patent, however, does not describe or disclose specific compounds in which each R^2 and R^5 is independently selected from a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom, as required by the amended claims. See Table 2, at column 9, in which R_2 and R_5 are both hydrogen for all six examples. Moreover, Applicants submit that one skilled in the art would not be motivated by the `692 Patent to replace the hydrogen groups with a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom with an expectation of increasing the effectiveness of

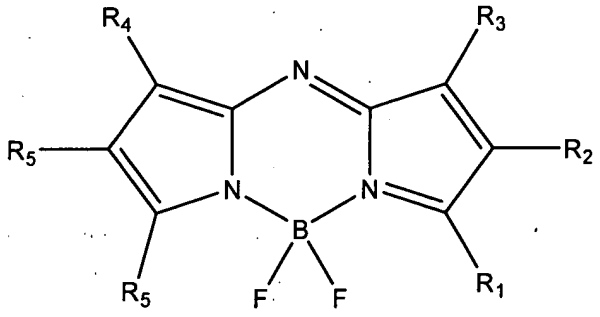
PDT by increasing the population of T1 and increasing the generation of ground state oxygen while limiting the non-radiative internal back-conversion from T1 to the ground state S0 and the inhibition of the photosensitizer triplet to ground state oxygen energy transfer. Rather, the `692 Patent is concerned with "microparticles incorporating a series of two or more fluorescent compounds having overlapping excitation and emission spectra, resulting in fluorescent microparticles with a desired effective Stokes shift." See column 1, lines 9 through 12.

As a result, the `692 Patent does not teach or suggest selecting R_2 and R_5 from the generic list of substituents at column 8, lines 49 through 64 to be a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom or modifying the specific examples in Table 2 to be a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom in order to produce microparticles exhibiting the desired effective Stokes shift, let alone selecting certain substitutes to produce compounds exhibiting increased population of T1 and increased the generation of ground state oxygen while limiting the non-radiative internal back-conversion from T1 to the ground state S0 and the inhibition of the photosensitizer triplet to ground state oxygen energy transfer.

For at least the foregoing reason, Applicants submit that the amended claims are non-obvious over the `692 Patent.

C. The JP Abstract

In making the obviousness rejection over the JP Abstract, the Examiner states that the JP Abstract discloses compounds of the formula



in which R_1-R_6 represent H, alkyl, alkoxy, alkenyl, alkoxycarbonyl, aryl, and heteroaryl. See the Official Action page 10.

Applicants note that the JP Abstract does not provide specific examples of the variables R_1-R_6 or describe or disclose specific compounds in which each R_2 and R_5 is independently

selected from a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom, as required by the amended claims.

Moreover, Applicants submit that one skilled in the art would not be motivated by the JP Abstract to utilize a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom at the R_2 and R_5 positions with an expectation of increasing the effectiveness of PDT by increasing the population of T1 and increasing the generation of ground state oxygen while limiting the non-radiative internal back-conversion from T1 to the ground state S0 and the inhibition of the photosensitizer triplet to ground state oxygen energy transfer. Rather, the JP Abstract is concerned with recording media facilitating short wavelength laser, high density recording and reproduction. See the abstract. Thus, the JP Abstract does not teach or suggest selecting R^2 and R^5 to be a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom in order to produce the desired recording media, let alone selecting certain substitutes to produce compounds exhibiting increased population of T1 and increased the generation of ground state oxygen while limiting the non-radiative internal back-conversion from T1 to the ground state S0 and the inhibition of the photosensitizer triplet to ground state oxygen energy transfer.

For at least the foregoing reason, Applicants submit that the amended claims are non-obvious over the `692 Patent.

D. Combinations of the `157 Patent, the `692 Patent, or the JP Abstract

Applicants respectfully submit that the amended claims are non-obvious over the combination of two or more of the `157 Patent, the `692 Patent, and the JP Abstract, since none of these references teaches or suggests compounds useful for PDT that include a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom at the R^2 and R^5 positions or provides the motivation necessary to make such modifications with the expectation of achieving compounds having desired properties. Indeed, each reference is concerned with different physical properties of the compounds. The `157 Patent is concerned with reducing triplet-triplet (T-T) absorption of compounds, the `692 Patent is concerned with producing microparticles exhibiting the desired effective Stokes shift, and the JP Abstract is concerned with producing recording media facilitating short wavelength laser, high density recording and reproduction.

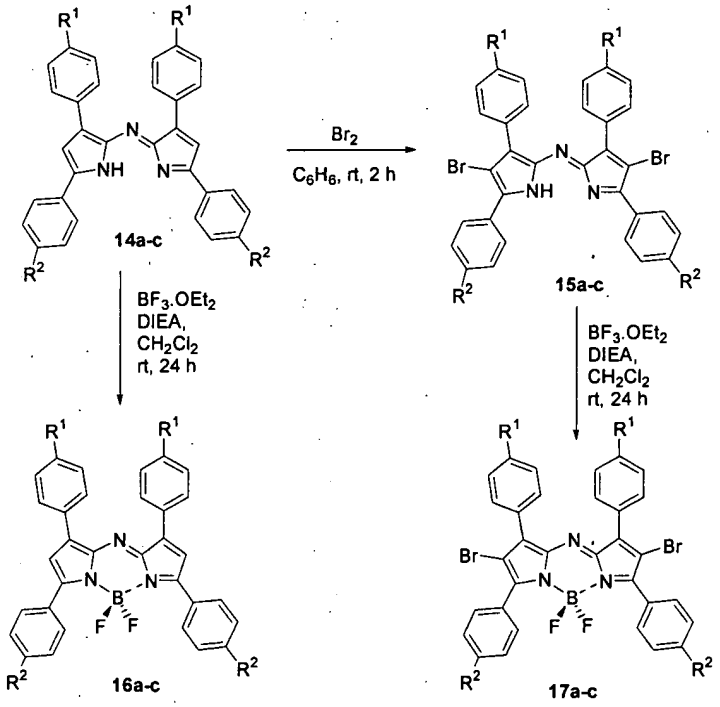
For at least the foregoing reasons, Applicants submit that the amended claims are non-obvious and patentable over the combination of two or more of the `157 Patent, the `692 Patent, and the JP Abstract.

D. Unexpected Results

Even if the Examiner contends that the `157 Patent, the `692 Patent, or the JP Abstract, alone or in any combination, renders the amended claims obvious, which Applicants maintain is not the case for at least the reasons discussed above, Applicants submit that the amended claims cover compounds exhibiting unexpected properties rendering them patentable. Namely, the compounds covered by the amended claims, in which R^2 and R^5 are a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom, are more efficacious for PDT relative to compounds of the prior art, such as compounds in which the substituent at the R^2 and R^5 positions is hydrogen.

The inventors have prepared compounds **16a-16c** and **17a** to **17c**, from the respective starting materials **14a** to **14c/15a** to **15c**, by following the methodology taught in Example 1 of the application as filed. Compounds **16a** and **17a** correspond to compound numbers **2a** and **4a** and Compounds **16b** and **17b** correspond to compound numbers **2b** and **4b** described in the application as filed.

Figure 1.



Compound	R^1	R^2
14a	H	H
14b	H	OCH_3
14c	OCH_3	H

Photosensitizer Spectroscopic Properties

The absorption spectra of **16a-c** and **17a-c** in aqueous formulated solution all show a strong $S_0 \rightarrow S_1$ transition with wavelength of maximum absorbance varying between 651 and 696 nm depending upon substituents (Table 1).

Table 1 Spectroscopic Absorbance Properties of **16a-c** and **17a-c**[a]

entry	comp.	λ max[b] / nm	λ max[c] / nm	λ max[d] / nm	λ max[e] / nm	fwhm / nm	ε[e] / M^{-1} cm^{-1}
1	**16a**	658	655	647	650	53[b] (49)[e]	79,000
5	**17a**	651	652	645	650	57[b] (47)[e]	79,000
2	**16b**	696	693	686	688	57[b] (55)[e]	85,000
6	**17b**	685	683	675	679	86[b] (57)[e]	75,000
3	**16c**	671	666	660	664	57[b] (57)[e]	78,000
7	**17c**	655	655	646	653	66[b] (57)[e]	80,000

[a] Concentration 5 x 10^{-6} M, rt. [b] H_2O / Cremophor EL(CrEL). [c] Toluene. [d] Ethanol. [e] Chloroform.

Comparisons of **16a-c** with their corresponding di-brominated derivatives **17a-c** show that the introduction of the heavy-atoms gives rise to a moderate hypsochromic shift ranging from 7 to 16 nm. Remarkably, the substitution of bromines onto the pyrrole rings results in only minor changes in the maxima or the shape of the absorption bands. For example, a comparison of **16a** and **17a** in aqueous solution show only a variance of 7 nm in their λ max values (Table 1, entries 1, 5). This demonstrates that the heavy-atom can be introduced without diminishing the advantageous absorption characteristics of the photosensitizers of the present invention.

The fluorescence properties of the sensitizers were examined in aqueous formulated solutions, toluene, ethanol and chloroform. Excitation of the compounds **16a-c** and **17a-c** in aqueous solutions at 630 nm all gave fluorescence bands which were mirror images of the absorbance spectra with Stoke shifts in the range of 22 to 38 nm (Table 2). The compounds **16a-**

c showed a range of high fluorescence quantum yields (Φ_f) measured in chloroform from 0.23-0.36 (Table 2). In comparison, the introduction of bromine directly into the core of the photosensitizer gave rise in each case to substantial reduction in fluorescence quantum yields for **17a-c** (Table 2, entries 5, 6, 7) indicating that, when a heavy atom such as bromine is directly substituted onto the central core of the photosensitizer, a heavy-atom effect can be induced which, depending upon other possible competing photophysical pathways, may translate into increased singlet oxygen production.

Table 2. Spectroscopic Fluorescence Properties of **16a-c** and **17a-c**[a]

entry	comp.	λ max[b] / nm	Stoke shift[b] / nm	λ max[c] / nm	λ max[d] / nm	λ max[e] / nm	Φ_f[e]
1	**16a**	683	25	676	669	672	0.34
5	**17a**	679	28	672	666	673	0.012
2	**16b**	727	31	717	715	715	0.36
6	**17b**	719	36	714	712	714	0.10
3	**16c**	701	30	693	697	695	0.23
7	**17c**	693	38	683	680	679	<0.01

[a] Concentration 2 x 10^{-7} M, rt. [b] H_2O / CrEL. [c] Toluene. [d] Ethanol. [e] Chloroform.

X-Ray Structure of 17a

The X-ray structure of **17a** provides solid state evidence for a brominated derivative, the patent application already providing a comparative crystal structure for a non-brominated example.

The introduction of heavy-atoms with large atomic radii into a photosensitizer can result in structural deformation to the planarity of the molecule. In order to assess whether structural deformation had occurred, the sensitizer structure imparted by the bromine atoms in **17a-c**. **17a** was crystallized. This was achieved by the slow room temperature evaporation of a toluene solution, in the monoclinic space group Cc (#9) with four molecules in the unit cell. A thermal ellipsoid drawing of **17a** is shown below:

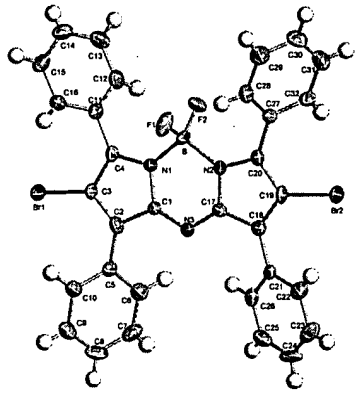


Figure 2. Perspective drawing of **17a.** Thermal ellipsoids drawn at 80% probability level.

In spite of the introduction of two bromine atoms onto the sensitizer **17a**, the planarity of the central 12-atom core of the molecule was preserved. The bromine bond angles from pyrrole ring 1 (N1/C1/C2/C3/C4) are -0.126(1) Å for Br(1) and 0.109(1) Å for Br(2) from planarity and for pyrrole ring 2 (N2/C17/C18/C19/C20) Br(1) is –0.415(1) Å and Br(2) is 0.004 Å. The angle of intersection of pyrrole ring 1 and pyrrole ring 2 is small at 4.2(2) degrees. The 12-atom plane of the central tricyclic structure shows the greatest deviation from this plane of 0.100(2) Å for the atom N(1). Unexpectedly, this structure compares favorably with the structure of non-brominated **16b** reported in Figure 3 of the application as filed, which has a comparable intersect angle of pyrrole ring 1 and pyrrole ring 2 of 4.1(3) degrees. Additionally, the central tricyclic 12-atom plane of **16b** shows the greatest deviation from this plane of 0.087(3) Å for the atom N(2). These results support the supposition that introduction of heavy atoms such as bromines at β-pyrrole position of **17a-c** could give rise to a more efficient population of the triplet state without causing an increase in non-radiative decay to the ground state.

Comparative Study of Singlet Oxygen Generation in Solution with Light > 600 nm

The ability to gain control over singlet oxygen production by exploiting the heavy-atom effect, was tested by carrying out a comparative singlet oxygen generation analysis. The study was undertaken by monitoring the reaction of the singlet oxygen acceptor 1,3-diphenylisobenzofuran (DPBF) with photosensitizer generated singlet oxygen by following the disappearance of the 410 nm absorbance band of DPBF at initial concentration of 5×10^{-5} M over a time period of 1 h. Each of the sensitizers **16a-c** were examined at a concentration of 5×10^{-6} M and compared to hematoporphyrin as a reference sensitizer. The pyrrole brominated

derivatives **17a-c** were examined at a lower concentration of 5 x 10^{-8} M and compared to the reference sensitizer methylene blue. Relative rates of oxygenation of DPBF by **16a-c** and **17a-c** versus hematoporphyrin and methylene blue were estimated by comparison of the rates of consumption of DPBF at the initial stages of each experiment. The standard sensitizers (hematoporphyrin and methylene blue) have singlet oxygen quantum yields of 0.65 and 0.50, respectively, in methanol.

Table 3. Comparative singlet oxygen generation of **16a**, **16b**, **16c**, hematoporphyrin at 5 x 10^{-6} M concentration.

Compound	**1***	**16a**	**16b**	**16c**
rel. rate	1	0.46	0.4	1.2

*hematoporphyrin

Table 4. Comparative singlet oxygen generation of **17a**, **17b**, **17c**, methylene blue at 5 x 10^{-8} M concentration.

Compound	**9***	**17a**	**17b**	**17c**
rel. rate	1	2.9	7.7	4.2

*methylene blue

A heavy-atom effect can be observed when R^2 and R^5 is an alkyl, cyclic or heterocyclic moieties with heavy atom substitutions, so that the heavy atom (such as bromine) is not incorporated directly on the central core of the photosensitizer. All of **17a-c** showed an increased efficiency of singlet oxygen generation in comparison to **16a-c**, even at a one-hundred fold lower concentration of 5 x 10^{-8} M. The dramatically enhanced singlet oxygen production levels of **17a-c**, when contrasted with **16a-c**, show that the inclusion of the heavy-atom as a substituent directly onto the central core of the photosensitizer results in singlet oxygen production without given rise to loss of excited state energy by internal radiationless transitions. No significant photobleaching of the sensitizers was observed during these experiments.

Light Induced Cytotoxicity Assay

Two different cell types were examined in the assay, MRC5-SV40 transformed fibroblast cells and HeLa cells. Varying concentrations of Cremophor EL formulated aqueous solutions of

the photosensitizers were incubated with the cells in the dark for 3 h. Subsequently, the culture medium was removed and fresh culture medium added to each well. The plates were irradiated using a light source of wavelength 600-750 nm delivering a light dose of either 8 or 16 J cm^{-2}. Following irradiation, the cells were incubated for a further 48 h at 37 °C, after which time, percentage cell viability was determined using a tetrazolium chlorimetric reduction assay. Dark toxicity of photosensitizers was determined by carrying out an identical experiment as described above except that the light irradiation step was omitted (0 J cm^{-2}). All assay experiments were carried out in triplicate and an average of the three individual runs are presented. Hematoporphyrin was used as a comparative standard control and was assayed according to previously documented procedures.

MRC5-SV40 cells displayed no determinable dark toxicity with **16b, 17b** or hematoporphyrin up to a concentration of 10^{-4} M, Table 5. In contrast, irradiation with 8 J cm^{-2} light dose showed a significant light induced toxicity with EC-50 values determined for **16b** and **17b** as 1.1 x 10^{-4} and 3.7 x 10^{-8} M, respectively (Table 5). The exceptional light induced toxicity of **17b** was very encouraging as this molecule contained the two bromine heavy atoms directly substituted onto the core of the photosensitizer.

As phototoxicity should be dependent upon light dose as well as photosensitizer concentration, the assay was repeated with a higher light irradiation of 16 J cm^{-2}. The higher light dose resulted in an improved EC-50 value for each of our studied photosensitizers with values obtained for **16b** at 1.7 x 10^{-5} M, and **17b** at 1.4 x 10^{-8} M (Table 5). Each of the tested photosensitizers, performed better at this light dose than the standard control hematoporphyrin (Table 5).

Table 5. *In vitro* EC-50 Assay Data for MRC5-SV40 Cells[a]

entry	comp.	EC-50 (M) / 8 J cm^{-2}	EC-50 (M) / 16 J cm^{-2}
1	**1***	6.3 (±3) x 10^{-5}	3.7 (±1) x 10^{-5}
3	**16b**	**1.1 (±1) x 10^{-4}**	**1.7 (±1) x 10^{-5}**
5	**17b**	**3.7 (±0.3) x10^{-8}**	**1.4 (±0.1) x10^{-8}**

[a] Standard deviation in brackets *hematoporphyrin

The data clearly portray how the compounds covered by the amended claims unexpectedly exhibit a remarkable spectrum of activity (from the micro- to nano-molar range) across these structurally related sensitizers. In the case of **17b**, exploitation of the heavy-atom effect *in vitro* is seen to be a viable method to control the excited triplet state population and singlet oxygen quantum yields, as well as to translate that control into significantly greater *in vitro* efficacy. **While in structural terms 16b and 17b only differ by the two bromine substituents, this gives rise to a divergence in efficacy by over a 1000 fold.**

A second study was carried out using the HeLa cell line. Photosensitizer dark toxicity was only observed at high concentrations for **16b,** with the most active compound **17b,** showing no observable dark toxicity in the tested concentration range (Table 6). A broad range of light induced cytotoxicity was also observed for the photosensitizer series in HeLa cells which was comparable to that observed in the MRC5-SV40 cell line. Determined EC-50 values for the series with a light dose of 16 J cm^{-2} showed that **16b** (3.1 x 10^{-5} M) was the least active in the assay, the best being **17b** (4.1 x 10^{-8} M) (Table 7). An improvement in efficacy for each photosensitizer was observed on increasing the light dose from 8 to 16 J cm^{-2}, again showing the expected light-dose response behavior. Again, the *in vitro* heavy-atom effect was clearly observed, when comparing EC-50 data for **16b** and **17b** at a light dose of 8 J cm^{-2}, there was over **a 1000-fold efficacy increase** while, at 16 J cm^{-2}, there was greater than **a 750 fold increase** (Table 6).

Table 6. *In vitro* EC-50 Assay Data for HeLa Cells[a]

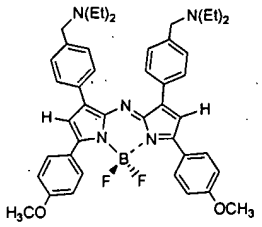
entry	compound	EC-50 (M) / 0 J cm^{-2}	EC-50 (M) / 8 J cm^{-2}	EC-50 (M) / 16 J cm^{-2}
1	**1**	none	3.3 (±2) x 10^{-5}	1.9 (±0.4) x 10^{-5}
2	**16b**	1.1 (±1) x 10^{-4}	**7.2 (±5) x 10^{-5}**	**3.1 (±1) x10^{-5}**
3	**17b**	none	**6.3 (±2) x 10^{-8}**	**4.1 (±3) x 10^{-8}**

[a] Standard deviation in brackets

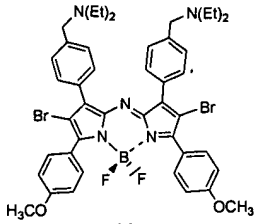
Further Examples of the Effect of Heavy Atom Substitution

The compounds in Figure 3 were synthesized and tested.

Figure 3. Further Heavy-Atom Efficacy Comparison.



18



19

The compounds including a heavy atom unexpectedly result in a very large differential in efficacy between the heavy-atom functionalized example (19), when compared to the non-heavy atom substituted derivative (18), with a **480 fold efficacy difference** recorded (Table 7).

Table 7. pKa in H_2O/CrEL,[a] Acid Enhanced Singlet Oxygen Generation[b] and *in vitro* MRC5 cell line EC_{50} assay data[c] for **18** and **19**.

Entry		pKa	$^1O_2$ acidic rate increase	EC_{50} x 10^{-6} (M) MRC5 cell	
				0 J cm^{-2}	16 J cm^{-2}
1	**18**	6.6	9.2	> 100	2.8
4	**19**	6.6	10.6	4.5 (± 0.7)	0.0058 (±0.003)

[a] pKa measured by fluorescence titration in H_2O/CrEL solutions. [b] rate of singlet oxygen generation in acidic DMF / rate in DMF. [c] light dose of either 0 or 16 J cm^{-2} with standard deviation in brackets.

Thus, compounds include covered by the amended claims, in which R^2 and R^5 are a heavy atom or an alkyl, cyclic, or heterocyclic moiety each substituted with at least one heavy atom, unexpectedly result in more efficacious PDT relative to the compounds of the prior art, in which R^2 and R^5 are hydrogen, by increasing the population of T1 and increasing the generation of ground state oxygen while limiting the non-radiative internal back-conversion from T1 to the ground state S0 and the inhibition of the photosensitizer triplet to ground state oxygen energy transfer. The compounds covered by the amended claims and the unexpected properties they posses are neither disclosed nor suggested in the prior art.

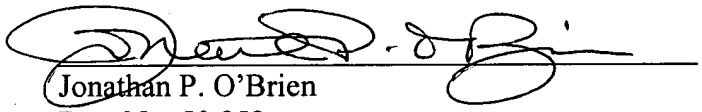
CONCLUSION

For at least the foregoing reasons, Applicants respectfully request that the Examiner reconsider and withdraw the anticipation and obviousness rejections; allow claims 27 and 37-58; and rejoin and allow claims 31 and 58-69.

Applicants are requesting an one month extension of time for responding to the Official Action dated March 27, 2006, and petition herewith under 37 CFR 1.136(a) to extend the period for filing this response and authorize the Director to withdrawal the requisite fee of $60.00 from Deposit Account Number 503654.

Respectfully submitted,
DONAL O'SHEA ET AL.
By: Miller, Canfield, Paddock and Stone, P.L.C.

JULY 27, 2006
Date



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